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Low-frequency seismic responses during microbial biofilm formation in sands

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This study investigates changes in low-frequency attenuation responses of sands during microbial formation of soft viscous biofilms, or extracellular polymeric substances (EPS). The resonant column experiments were conducted with two model bacteria *Shewanella oneidensis* MR1 and *Leuconostoc mesenteroides*, while monitoring changes in the wave velocities and damping ratios associated with EPS formation in sands. The results show that the accumulation of soft, viscous EPS hardly changes the wave velocities, both the shear and flexural modes. By contrast, the low-frequency attenuations, both torsional and flexural damping ratios, show significant increases with the accumulation of highly viscous EPS. It is found that the contribution of EPS to seismic responses of water-saturated sands is mainly limited to the pore fluid component, causing additional energy dissipation during wave propagation, but with no or minimal impact on skeletal stiffness or no involvement in seismic stress transfer. With these unique and unprecedented low-frequency seismic data of biofilm-associated sands, the results suggest that the formation and accumulation of soft, viscous EPS or biofilms by bacterial activities can be detected by monitoring seismic attenuation and can also alter the seismic attenuation responses of sands, such as the case under earthquake loading or blast-induced compaction.

Keywords: EPS formation/low-frequency seismic characteristic/small-strain dynamic property

Notation

C_c	coefficient of curvature
C_u	uniformity coefficient
D_{10}	particle size at which 10% of the particles are finer
D_{50}	particle size at which 50% of the particles are finer
D_f	flexural damping ratio (%)
D_s	shear damping ratio (%)
E	void ratio
E_{flex}	measured Young's modulus of the specimen (kPa)
G	measured shear modulus of the specimen (kPa)
G_d	dry shear modulus of soil (kPa)
G_{sat}	saturated shear modulus of soil (kPa)
K_d	bulk modulus of the solid framework of sand grains (kPa)
K_{EPS}	bulk modulus of the mixture of water and EPS
K_f	bulk modulus of the fluid (kPa)
K_m	average bulk modulus of the sand minerals (kPa)
K_{sat}	bulk modulus (kPa)
S_{EPS}	pore saturation of EPS
V_f	flexural wave velocity (m/s)
V_p	compressional wave velocity (m/s)
V_s	shear wave velocity (m/s)
γ	shear strain
ρ_f	bulk density of the pore fluid (kg/m ³)
ρ_m	bulk density of the sand minerals (kg/m ³)
ρ_{sat}	saturated density (kg/m ³)

Introduction

Microbial activities and products have garnered huge interest because these can spontaneously alter soil properties and hence, can be exploited as a means for soil modification and improvement. In particular, bacteria readily produce biofilms, which refer to the mixture of bacterial cells and extracellular polymeric substances (EPS). Previously, it has been reported that biofilms associated with EPS reduce porosity and permeability (Abbasi *et al.*, 2018; Bouwer *et al.*, 2000; Dunsmore *et al.*, 2003) and often increase strength (Karimi, 1998; Martin *et al.*, 1996). Accordingly, bio-grout using EPS or biopolymers is considered for soil erosion control, hydraulic barriers, and enhanced oil recovery (Cho and Chang, 2018; Choi *et al.*, 2020; Ham *et al.*, 2018; Kim *et al.*, 2019; Lappan and Fogler, 1996; Rebata-Landa and Santamarina, 2012; Tran *et al.*, 2019). Such microbial ground modification approaches with bacterial treatments inevitably involve injection of solutions containing bacteria and/or nutrients to stimulate in situ bacterial activities in subsurface.

Meanwhile, monitoring of in situ bacterial activities and their by-products in fields is critical for the successful field implementation of bacterial treatment. Seismic survey methods using P- and S-waves are proposed as a way to detect spatial and temporal changes associated with EPS production in subsurface. The frequency range relevant to the field seismic surveys, such as downhole survey, spectral analysis of surface wave (SASW),

multichannel analysis of surface waves (MASW) and cross-hole tomography, is generally less than 100 Hz. By contrast, most of the previous studies on seismic monitoring of microbial biofilms and EPS have been conducted at a high frequency range from kHz to MHz (e.g. Davis *et al.*, 2010; DeJong *et al.*, 2006, 2011; Kwon and Ajo-Franklin, 2011, 2013; Noh *et al.*, 2016). As a result, those studies have consistently reported that the production of biofilms or EPS in sands increases seismic attenuation but have no or minimal effect on P- and S-wave velocities (Kwon and Ajo-Franklin, 2013; Ta *et al.*, 2018). However, it is fairly challenging to infer the low-frequency field seismic responses using the data obtained at high frequency, owing to the dispersive characteristics in velocity and attenuation in granular and porous media (Priest *et al.*, 2005; Santamarina *et al.* 2001; Winkler, 1986). No or only limited attempts have been made to examine the changes in low-frequency seismic responses during EPS formation; to date, no measured data have been reported.

Therefore, this study explored the changes in shear and flexural wave velocities and attenuations (or damping ratios) in a low frequency regime, less than 100 Hz during bacteria EPS formation. Specifically, experiments were performed, in which the model biofilm-producing bacteria, *Leuconostoc mesenteroides* and *Shewanella oneidensis* MR1, were cultured in water-saturated sands and stimulated to produce EPS while monitoring the seismic responses in shear and flexural modes by using the resonant column (RC) device. This study presents unique and unprecedented experiment data which allow examination of the feasibility of seismic monitoring of bacterial EPS formation at the frequency directly applicable to field seismic surveys.

Materials and methods

Host sand

Fine silica sand (Ottawa F110) was used as the host soil in this study. This sand is mainly composed of quartz (>99%) and has a grain density of 2.65 kg/m³. It consists of rounded to sub-angular particles with the grain size ranging from 50 to 360 µm. This sand has these properties: minimum void ratio $e_{\min} = 0.53$, maximum void ratio, $e_{\max} = 0.85$, $D_{50} = 0.12$ mm, $D_{10} = 0.081$ mm, $C_u = 1.62$ and $C_c = 0$; various physical properties of Ottawa F110 sand can be further found in Santamarina and Cho (2001) and Cho *et al.* (2006).

Model bacteria and growth media

Two model bacteria were selected for this study: *Leuconostoc mesenteroides* (ATCC14935) to form polysaccharidic insoluble biopolymer called dextran and *Shewanella oneidensis* MR1 (ATCC700550) to form biofilms, respectively (Lappan and Fogler, 1994; Naessens *et al.*, 2005; Thormann *et al.*, 2004). There were two experimental runs conducted: one with *L. mesenteroides* and the other with *S. oneidensis* MR1. Both bacteria can produce insoluble extracellular polymeric substances (EPS); hereafter, the term EPS is used in this paper to refer to gel-like soft insoluble biopolymer products produced by the model bacteria (in this study), for simplicity.

Table 1 shows the defined growth media for the model bacteria *L. mesenteroides* and *S. oneidensis*. The growth medium for *L. mesenteroides* contains yeast extract, sucrose and a phosphate buffer, following the previous studies (Kwon and Ajo-Franklin, 2011; 2013 Lappan and Fogler, 1994). Yeast extract provides carbon and nitrogen sources for bacterial growth. The polysaccharidic EPS called dextran is produced by linking glucoses with glucosidic bonds through the extracellular breakdown of sucrose into glucose and fructose (Lappan and Fogler, 1994). The model bacteria *L. mesenteroides* are facultative anaerobes, thus, it can ferment regardless of the presence of oxygen. Using the defined growth medium, *L. mesenteroides* were aerobically cultured in a batch at room temperature and atmospheric pressure. Thereby, the model bacteria produced insoluble dextran in a gel phase over a short time period and that insoluble dextran became visible within 24 h, as demonstrated by previous studies (Kwon and Ajo-Franklin, 2011; 2013; Padmanabhan *et al.*, 2003). Figure 1 shows a highly viscous, ductile and gel-like bacterial dextran produced by *L. mesenteroides*.

S. oneidensis MR1 strain was chosen as the model bacterium in this study because they can grow both anaerobic and aerobic conditions, and the biosafety level is Class 1. *S. oneidensis* MR1 is a gram-negative facultative bacterium found in marine sediments. *Shewanella* species can form biofilms and reduce a broad spectrum of metals and organic compounds (Venkateswaran *et al.*, 1999). The growth media for *S. oneidensis* MR1 contain tryptic soy broth (TSB; Sigma-Aldrich Co), potassium phosphate monobasic (KH₂PO₄), potassium phosphate dibasic (K₂HPO₄) and sodium fumarate dibasic (C₄H₂Na₂O₄) (Table 1), and they were used to

Table 1. Compositions of the defined growth media for *L. mesenteroides* and *S. oneidensis*

Bacteria	Compound	Concentration: g/l	Molarity: mM	Density: g/cm ³	Viscosity: mPa s
<i>L. mesenteroides</i>	Yeast extract	10	–	1.030	1.09
	Sucrose	15	43.82		
	Monobasic – KH ₂ PO ₄	13.6	100		
	Dibasic – K ₂ HPO ₄	17.4	100		
<i>S. oneidensis</i>	TSB	60	–	1.053	1.23
	Sucrose	15	43.82		
	Monobasic – KH ₂ PO ₄	13.6	100		
	Dibasic – K ₂ HPO ₄	17.4	100		
	Sodium fumarate – C ₄ H ₂ Na ₂ O ₄	1.6	14		

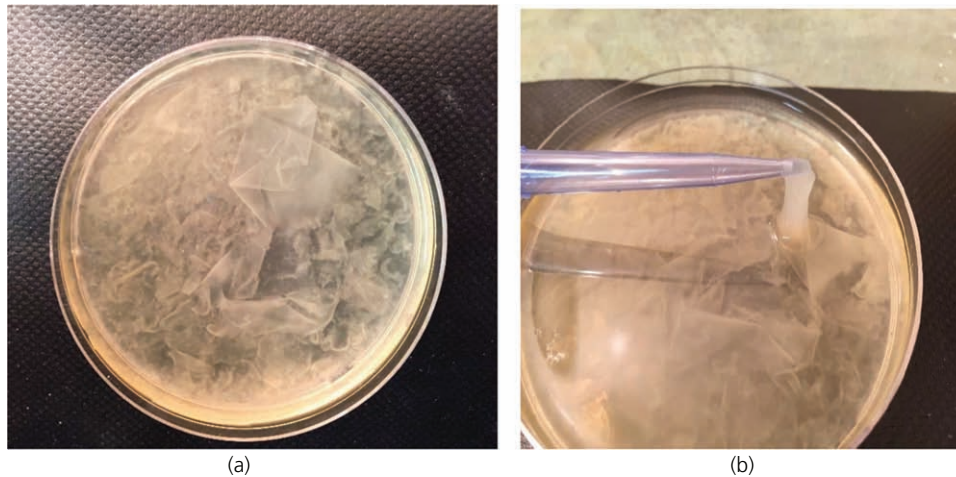


Figure 1. (a) Gel-like bacterial dextran, aerobically produced by *L. mesenteroides*, and (b) the gel-like texture of dextran

grow bacteria and stimulate their biofilm (EPS) formation. Note that the viscosity and density of the growth medium for *L. mesenteroides* and *S. oneidensis* MR1 are close to those of pure deionised water, as shown in Table 1. Therefore, the seismic responses of the soils containing these fluids (growth media) hardly differ from the ones containing pure water.

Specimen preparation

Dense sand specimens were prepared as they would minimise volume changes by confinement. Dry sand was pluviated through a funnel and tamped every 2 cm within a sample mould. This mould was lined with a latex membrane held in place by applying a vacuum between the mould and the membrane. The cylindrical specimens were 70 mm in diameter and 140 mm long, with the initial void ratio $e = 0.53$ – 0.54 and the relative density D_r greater than $\sim 97\%$ for the both experimental runs. A vacuum of ~ 10 – 15 kPa was applied to the specimen through the base pedestal to allow the removal of the specimen mould and the assembly of the RC drive system. Additional membrane was applied to the outside of the specimen (double membranes) with a layer of vacuum grease in between to mitigate the migration of compressed air to the specimen (see Figure 1(a) in the online supplementary material). The specimen was surrounded by a water-filled cylinder to apply the confining stress with water (Figure 1(b)). After the drive plate was mounted onto the top cap of the specimen (Figure 1(c)), the cell was fitted and an air pressure of 40 kPa was applied to the cell as a seating confining pressure while simultaneously releasing the vacuum (Figure 1(d)).

Bacterium inoculation, specimen saturation and continuous feed mode with growth media

Carbon dioxide (CO_2) was first flushed through the specimen for ~ 10 min to displace air inside the specimen because carbon dioxide is readily dissolved in water and aid in water saturation.

Each bacterial solution of *L. mesenteroides* and *S. oneidensis*

MR1 was prepared by aerobically culturing in the prepared growth media for ~ 24 h under an ambient temperature of ~ 22 – 24°C . The plate cell count tests estimated the bacterial cell density in the bacterial cultures to be $\sim 10^6$ – 10^7 cells/ml after 24 h. Then, the bacterial culture of 20 ml was mixed with the fresh growth medium of 200 ml and this mixture was used as the inoculum to be injected into the specimen. The inoculum of 150 ml, of which the cell density was $\sim 10^5$ – 10^6 cells/ml, was initially injected into the specimen at a constant flow rate of 10 ml/min using a syringe pump. The specimen was then left for 12 h to allow the bacteria attachment to the sand grains and further cell growth (Abdel *et al.*, 2010; Davis *et al.*, 2010; Kim and Fogler, 1999).

Thereafter, to achieve a fully saturated condition, the fresh growth medium with no bacterial cell was flowed into the soil specimen through the bottom platen. Figure 2 shows the flow-through experiment setup. During such upward flow, the fresh growth medium was flushed through both chambers of the pressure transducer, all valves, tubing and the flow pump to ensure that the system was fully saturated with water. The backpressure was set at 20 kPa and the cell pressure was then increased to 120 kPa to maintain the mean effective stress of 100 kPa. After 24 h of flowing the fresh media, the cell pressure and backpressure (or pore fluid pressure) were elevated in stages to 210 and 110 kPa, respectively, maintaining the effective stress equal to 100 kPa. This backpressure ensured no gas bubble generation in the soil specimen. Skempton's B-value parameter was measured during this process to evaluate the initial saturation of the specimen. The B-value of 0.99 was attained in both experiments with *L. mesenteroides* and *S. oneidensis*, which confirmed the full saturation of the specimens.

Finally, the fresh media were then fed at 0.2 ml/min to commence the continuous feed mode. This flow rate was chosen so as to satisfy a laminar flow regime with Reynolds number (Re) less

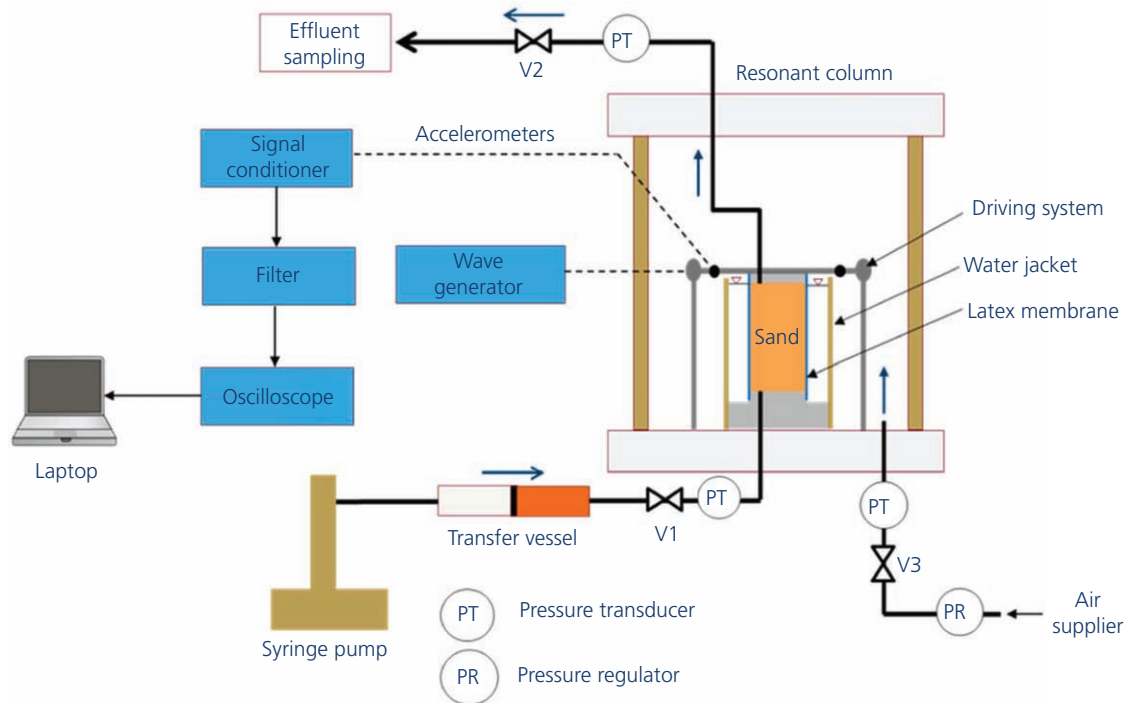


Figure 2. The schematic diagram of the resonant column experiment

than 1 ($Re = 0.25$ in this study) and not to cause local accumulation of EPS close to the injection port. The continuous feed mode was kept over the course of the experiments which lasted 20 days. This method was designed to simulate the naturally occurring processes of biofilm formation in soils (Figure 2). It was extremely challenging to quantify the amount of EPS accumulating within the specimens because the kinetics of EPS formation, by the chosen model bacteria and the detached EPS that flowed out during the continuous feeds, were unknown. This study unfortunately lacks information on the EPS quantity accumulated. The bacterial EPS flowed out of the outlet fluid port, which suggests at least no local clogging of EPS close to the nutrient injection port. During the experiments, the bacterial EPS production was visually confirmed through separately culturing part of the effluent samples. A small fraction of effluent cultures was also scanned using a FEI Quanta 200 scanning electron microscope to observe qualitatively the morphology of cells and EPS, in which a low-vacuum mode was applied to minimise changes to the dextran structure during scanning. Besides, the purity of the bacterial cultures in the specimens was confirmed by analysing the 16S ribosomal RNA (16S rRNA) genetic sequence of the cultured bacteria in the effluent samples. Microbial biodegradation of latex rubbers can cause ruptures in latex membranes and hence, a sudden increase in pore pressure (Rose and Steinbüchel, 2005). However, there was no sudden change in pore pressure observed in the current study, which indicated that no significant degradation took place with the model bacteria for the time scale of the experiments.

RC apparatus and measurements

The standard Stokoe RC was modified to apply flexural excitation as well as torsional excitation, as shown in Figure 2. The torsional frequency ranges from 17 to 25 Hz for soft clay soils (Hardin and Drnevich, 1972) and up to 400 Hz for stiff cemented sands (Avramidis and Saxena, 1990). This falls within the frequency range employed in marine seismic surveys. The RC can determine the small-strain mechanical properties, for example, $\gamma = 10^{-6}$ – 10^{-3} using the equations for the fixed-free type RC (Clayton *et al.*, 2009). Also, the flexural excitation can be beneficial because compressional wave velocity can be computed from the flexural and torsional resonance frequencies (Cascante *et al.*, 1998). Furthermore, the device was modified to allow fluid flows through the soil specimen and continuous nutrient feeds during the experiments. Details of the RC test used in this study, including the basic principle, device calibration and data reduction, are provided in the online supplementary material.

Methodology

Torsional or flexural vibration was excited to the top of the specimen by applying a sinusoidal voltage to the drive coils. This produced an oscillatory motion at the magnet-coil drive attached to the top of the specimen due to the interaction between the electromagnetic field in the coils and the fixed magnets attached to the drive plate. The drive frequency increased incrementally from 0.2 to 170 Hz (frequency sweep) while the shear strain range was kept consistently between 10^{-6} and 10^{-5} below the elastic threshold strain. The resonant frequency of the specimen was found by monitoring the response of

an accelerometer installed at the drive plate during such frequency sweeping. By plotting the output voltage of the accelerometer against the applied frequency, the resonant frequency could be identified easily. This RC measurement was repeated every 24 h for the entire experiment period.

System setup

The schematic diagram of the instrumentation used in the RC test is illustrated in Figure 2. The input signal to the driving coils was generated by a signal generator (Agilent 33509B). The response of the specimen was measured with two accelerometers (PCB 353B51). The output signals were conditioned and amplified by using a signal conditioner (PCB 482C27) and a filter (Krohn-Hite 3944) and then recorded by a digital oscilloscope (Agilent DSOX2024). A transparent polycarbonate transfer vessel was connected to a syringe pump (500D, ISCO-Teledyne) and used to inject the fresh growth media into the specimen as the syringe pump provided water feed at a controlled flow rate. The cell pressure was applied by using a high-pressure air supply system and the pore fluid pressure was controlled by a back-pressure regulator.

Data analysis and reduction

Based on the resonant frequencies of the torsional and flexural excitation modes, the shear wave velocity V_s , shear damping ratio D_s , flexural wave velocity V_f and flexural damping ratio D_f were obtained. The resonant frequency was determined to be the frequency with the highest voltage output from the accelerometers. Once a frequency spectrum was established, the half-power bandwidth method was used to determine the damping ratio (Drnevich and Richart, 1970; Vucetic and Dobry, 1991). Interpretation of the acquired seismic responses assumed a uniform EPS distribution over the specimen. Details of this data analysis and reduction methods are further described in the online supplementary material.

Results and discussion

Shear wave velocity and flexural wave velocity

Shear wave velocity V_s and flexural wave velocity V_f can be directly obtained from the torsional and flexural vibrations of the specimens, respectively. Figure 3 shows changes in V_s and V_f for the two experiments with different bacteria. The initial V_s and V_f values are in consistent ranges with the previously reported values in literature (e.g. Cascante *et al.*, 1998) though in the current study, the values are faster by ~10% owing to the angular characteristics of Ottawa F110 sand grains. This validates the RC measurements in the current study. V_s exhibits a slight decrease from 279 to 262 m/s by 6.1% in the experimental run with *L. mesenteroides* (Run 1) and from 282 to 273 m/s by 3.2% in the experimental run with *S. oneidensis* MR1 (Run 2). V_f also decreases from 398 to 376 m/s by 5.5% with *L. mesenteroides* and from 400 to 391 m/s by 2.4% with *S. oneidensis*, respectively. It is observed that the bacterial EPS accumulation causes no increases but only slight reductions (less than ~6%) in the wave velocity.

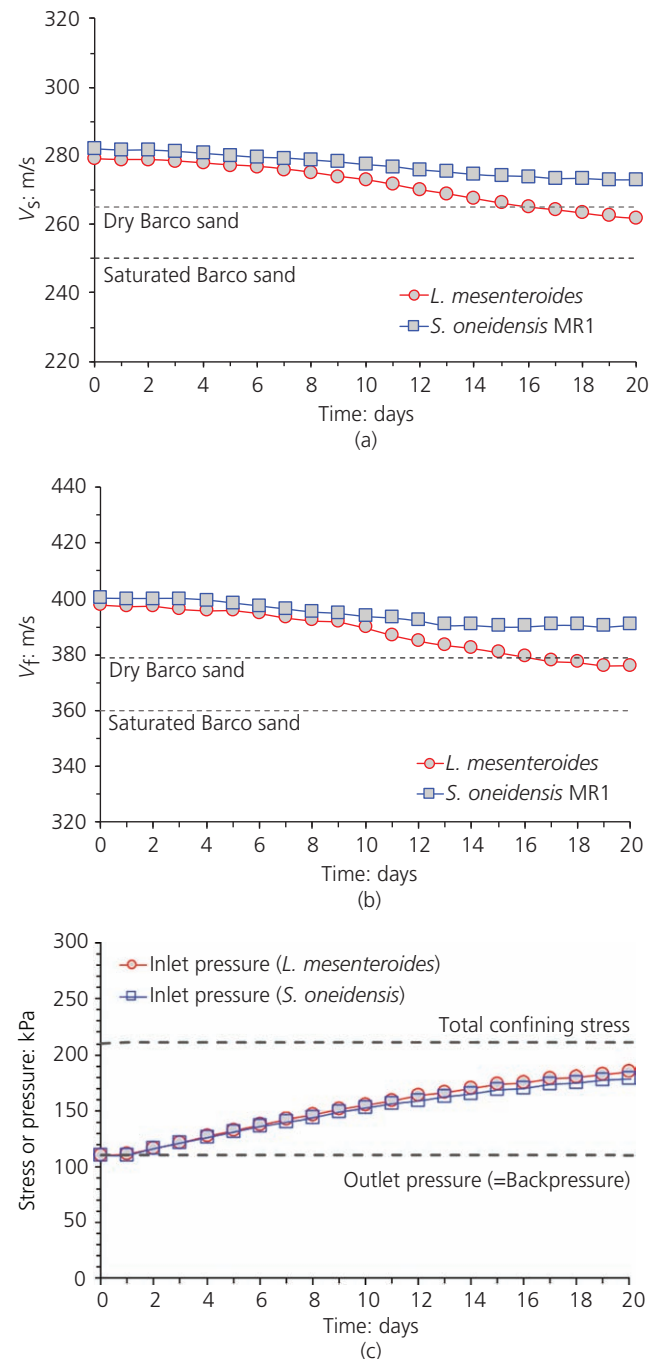


Figure 3. Changes in (a) shear wave velocity V_s , (b) flexural wave velocity V_f and (c) pore pressures over the course of experiments during EPS formation by *L. mesenteroides* and *S. oneidensis* MR1. Over the course of both experimental runs, the total confining stress and the pore pressure at the outlet (or backpressure) were kept constant at 210 and 110 kPa, respectively. The V_s and V_f values of dry and saturated Barco sands ($D_{50} = 0.44$ mm), measured using RC by Cascante *et al.* (1998), are superimposed for comparison with the current study's initial baseline measurements

Over the course of the experiments, the fresh media were kept continuously fed at a constant flow rate of 0.2 ml/min for continuous bacteria feeding. Accumulation of EPS in the pore spaces decreases hydraulic conductivity and thus, it results in an increase in the pressure gradient across the specimen to maintain the constant flow rate. Expectedly, an increase was observed in the inlet pressure from 110 to ~180 kPa while the backpressure regulator kept the outlet pressure constant at 110 kPa, as shown in Figure 3(c). This was consistently observed in both experimental runs. On the other hand, those gradual and continuous increases in the pressure gradient across the specimens support that the microbial activities and EPS production by the inoculated bacteria were consistently active over the course of the experimental runs.

The inlet pressure was not measured directly within the specimen but monitored at one location of the injection side tubing connected to the bottom platen. It is worth noting that the clogging also took place in the tubing, causing considerable pressure drop within the flow plumbing line before entering the specimen. Therefore, the accurate estimation appears unpalatable, but it is presumed that the effective confining stress also gradually decreased during bacterial growth and EPS accumulation. Therefore, the slight decreases in the shear and flexural wave velocities are attributable to the reduction in the effective confining stress, not relevant to the bacterial EPS accumulation. Conclusively, test results in the current study clearly indicate that biofilm and EPS formation hardly alters, to any significance, the grain contact stiffness.

Impact of EPS on V_p and V_s of water-saturated sand estimated by using Gassman model

In the flexural mode, bending produces an unequal strain field across a cylindrical specimen, therefore on one side, the specimen is in compression and on the other, extension. In saturated sand, water is free to move across the specimen to equilibrate the excess pore pressure caused during bending (Cascante *et al.*, 1998). Therefore, this implies that the Young's modulus obtained using the flexural excitation represents the dry skeleton only. Whereas, as the movement of a compressional wave through a specimen creates an even strain field in the cross-section, excess pore pressure cannot dissipate and the stiffness of both the water and the frame affects compressional wave propagation. To derive the saturated compressional wave velocity of the sediments in the RC test, the stiffness of the water must be factored into the flexural mode results (Cascante *et al.*, 1998).

Compressional wave velocity V_p and shear wave velocity V_s in saturated sand can be expressed using the shear modulus G_{sat} and bulk modulus K_{sat} and saturated density ρ_{sat} :

$$1. \quad V_p = \left(\frac{K_{sat} + \frac{4}{3} G_{sat}}{\rho_{sat}} \right)^{1/2}$$

$$2. \quad V_s = \left(\frac{G_{sat}}{\rho_{sat}} \right)^{1/2}$$

As fluid does not have shear modulus, the saturated shear modulus G_{sat} remains the same as that of the dry frame, $G_{sat} = G_d$. The saturated bulk density is the following:

$$3. \quad \rho_{sat} = (1 - \phi)\rho_m + \phi\rho_f$$

where ρ_m and ρ_f are the bulk density of the sand minerals and pore fluid, respectively; and ϕ is the porosity of the sand.

Gassmann (1951) derived an equation to compute the bulk modulus of a saturated sand K_{sat} using the bulk moduli of the constituent parts:

$$4. \quad K_{sat} = K_d + \frac{\left(1 - \frac{K_d}{K_m}\right)}{\frac{\phi}{K_f} + \frac{1 - \phi}{K_m} - \frac{K_d}{K_m^2}}$$

where K_f is the bulk modulus of the fluid; K_m is the average bulk modulus of the sand minerals; K_d is the bulk modulus of the solid framework of sand grains which can be calculated from the elastic properties obtained from the RC (Priest *et al.*, 2005):

$$5. \quad K_d = \frac{E_{flex} \times G}{3(3G - E_{flex})}$$

where E_{flex} and G are the measured Young's modulus and shear modulus of the specimen (equivalent to the frame moduli), respectively.

The bulk modulus (K_f) and density (ρ_f) of the multiple-phase pore fluid can be expressed as follows:

$$6. \quad K_f = \left[\sum_{i=1}^n \frac{S_{fi}}{K_{fi}} \right]^{-1}; \quad \sum_{i=1}^n S_{fi} = 1; \quad \rho_f = \sum_{i=1}^n S_{fi}\rho_{fi}$$

where n is the number of fluid components, K_{fi} , ρ_{fi} and S_{fi} are bulk moduli, density and saturation of the i -th fluid component present in the pores, respectively.

In general, there are several pore-scale mechanical models that can be used to describe the contribution of biofilms to the whole frame stiffness: the contact-cementation model, the grain-coating model, the load-bearing model and the pore-filling model. If a

biofilm or EPS contributes to the skeletal stiffness either as a cementing agent or as an additional mineral grain, it would be described by using the contact-cementation, grain-coating, or load-bearing models. On the other hand, if EPS is introduced as part of the pore fluid and it only contributes to the bulk modulus of the pore fluid, it corresponds to the pore-filling model. Therefore, the contribution of EPS to the sand frame moduli in the former models or the latter pore-filling form will result in different effects on the wave velocities and moduli of sand.

Several studies have shown that the biofilms first originate from the surface of the mineral grains and then spread both vertically and horizontally (Klausen *et al.*, 2003). Due to lack of pore spaces, biofilms prefer to expand along the surface, which results in their interconnection at the contacts of grains. At the mature stages of a biofilm, it coats the grain surfaces and bridges the mineral grains. Meanwhile, it is well-recognised that biofilms are soft matters with very low stiffness. Kim *et al.* (2017) reported the Young's modulus of biofilms produced by *S. oneidensis* MR1 to be ~33–38 kPa. Körstgens *et al.* (2001) determined that the Young's modulus of biofilms produced by *Pseudomonas aeruginosa* was 6500 Pa. Jeon *et al.* (2017) measured the shear modulus of EPS dextran produced by *L. mesenteroides* to be ~0.1 Pa. Klapper *et al.* (2002) and Mayer *et al.* (1999) measured the shear modulus of biofilms for the same bacteria to be ~50–70 Pa. Therefore, it can be assumed that biofilms or EPS hardly plays a role as the cementing, coating, or load-bearing inclusions in sands. Instead, the pore-filling form can be assumed prevalent.

According to the pore-filling model, when the EPS saturation S_{EPS} increases, the skeletal moduli K_d , E_d , G_d , and G_{sat} and the porosity ϕ remain constant. The bulk modulus of pore fluid that is the mixture of water and EPS K_{EPS} also remains fairly unchanged because K_{EPS} is expected to be equivalent to that of water. Thereby, the saturated moduli K_{sat} and G_{sat} remain nearly constant. By contrast, the fluid density ρ_f increases, and this in turn reduces V_p and V_s of the system. Therefore, assuming that the EPS only contributes to the pore fluid part in the pore-filling form, it can be expected that the velocity reductions in V_p , V_s and V_f are mainly attributable to the increase in bulk soil density associated with the accumulation of EPS that are denser than water.

Let us examine the impact of EPS on wave velocity by using the Gassman model and the initial V_f and V_s prior to EPS production. For calculating the compression wave velocity V_p (Equation 1) in RC tests, individual values of K (Equations 4 and 5), G (Equation 2) and ρ (Equation 3) of all three of the components, such as EPS, sand and media, are required. Although the EPS quantity produced within the specimens is not measured in the RC tests, the Gassman model estimation (Equation 5) allows assessment on variations in V_p and V_s with accumulation of EPS in sand. The Ottawa sand used in the experiments in the current study can be considered as quartz with the values of K , G and ρ found in

Helgerud *et al.* (1999). The properties of the growth media are assumed to be the same with those of water. Guided by the previously measured elastic moduli of biofilms and EPS (e.g. Jeon *et al.*, 2017; Kim *et al.*, 2017; Klapper *et al.*, 2002; Körstgens *et al.*, 2001; Mayer *et al.*, 1999), the parameters and values used in the analysis (in the current study) are determined, as listed in Table 2. Besides, the density of EPS was assumed the same with the value of dextran and it was measured as 1.47 g/cm^3 by using the pycnometer method. In the pore-filling model, the bulk modulus of dry skeletal frame K_d in Equation 5 remained unchanged and it can be calculated using the initial values of E_{flex} and G which were measured from the RC test prior to EPS formation.

Figure 4 shows the changes in V_p and V_s as a function of EPS saturation S_{EPS} (i.e. pore volume fraction of EPS, defined as the EPS volume divided by the pore volume), computed based on the Gassmann model. The pore-filling model predicts slight decreases in V_p and V_s with EPS growth in sand. The inclusion of EPS as part of the pore fluid increases the bulk density of the sand and this causes the reductions in V_p and V_s while the elastic moduli of the water-saturated sand K_{sat} and G_{sat} stay constant. In the model, the properties of EPS produced by *L. mesenteroides* and *S. oneidensis* are assumed to be the same due to the limited information. The difference originated from the initial packing

Table 2. Parameters of the biofilm-associated and fluid-saturated sand constituents

Material	Density, ρ : g/cm ³	Viscosity: mPa	Bulk modulus K : GPa	Shear modulus G : GPa
Quartz	1.72	–	36.6	45
Biofilm	1.5	–	6.5×10^{-6}	6×10^{-8}
Fluid	1	–	2.25	0

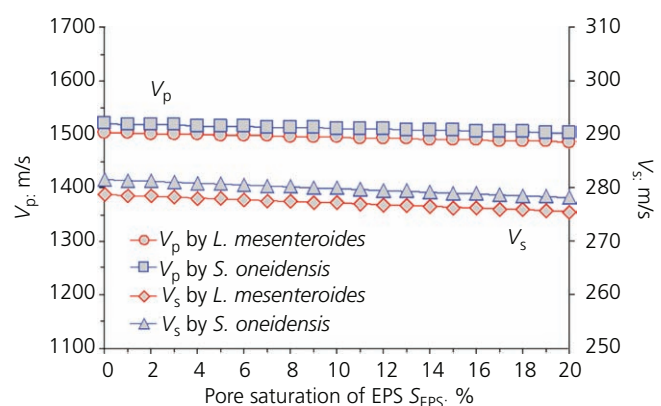


Figure 4. Variations in compressional wave velocity, V_p , and shear wave velocity, V_s , with EPS pore saturation, S_{EPS} , estimated by using Gassmann's model

properties of sand, such as porosity, density, E_{Flex} , and G obtained from the test. Thus, the variations in V_p and V_s for both bacteria plot parallel.

Low-frequency attenuation characteristics: damping ratios

In this study, the damping ratio was determined to examine low-frequency attenuation characteristics by using the half-power bandwidth method, which involves quantifying the width of the frequency response curves in both torsional and flexural modes. Figure 5 shows the variations in shear and flexural damping ratios during the EPS accumulation. The initial shear and flexural damping ratios are placed in consistent ranges with the previously reported values in literature (e.g. Cascante *et al.*, 1998), which validate the RC measurements in the current study. The results show significant increases in the shear damping ratio D_s ; D_s increases from $\sim 0.6\%$ to $\sim 2\%$, by $\sim 210\%$ for Run 1 with *L. mesenteroides* and from $\sim 0.5\%$ to $\sim 1.5\%$, by $\sim 165\%$ for Run 2

with *S. oneidensis* MR1 due to the EPS accumulation (Figure 5(a)). The similar increasing trend is also seen with flexural damping ratio D_f , but at a smaller rate compared to the increase in shear damping. D_f increases from $\sim 3.5\%$ to $\sim 6\%$ by 52% for Run 1 with *L. mesenteroides* and from $\sim 3\%$ to $\sim 4.5\%$, by 42% for Run 2 with *S. oneidensis* MR1, due to the existence of highly viscous, ductile, gel-like EPS in the specimens. The damping ratio values become nearly constant after 12 days of the steady increases. This is possibly associated with the shear detachment of old EPS which flows out of the specimen, while fresh newly produced EPS forms within the specimen.

The torsional and flexural excitations were imposed at small strain less than the elastic threshold strain ($<10^{-5}$) and in low frequency ranges of 0.2–170 Hz. In this small-strain and low-frequency regime, the energy dispersion in fluid-saturated soils is primarily due to the viscous dissipation and not by the internal frictional losses (Winkler *et al.*, 1979). In clean water-saturated porous media, local fluid flows of water in pores and cracks are the primary contributor to energy loss (or damping), in which the energy is dissipated by the cyclic movement of interstitial pore fluid around deforming grain contacts (Cascante, 1996; Johnston *et al.*, 1979; Jones, 1986; Murphy *et al.*, 1986). This flow-induced mechanism, called the squirt-flow model, has a primary role in determining the damping ratio at small strain levels (less than $\gamma = 10^{-5}$) and at a low frequency range (less than 200 Hz) (Kwon and Ajo-Franklin, 2013; Mavko and Nur, 1979; Mavko *et al.*, 2009; Noh *et al.*, 2016).

Meanwhile, the biofilms or EPS formed in the water-saturated sands contributed to additional energy loss and thus, increased the attenuation and damping ratio. Such energy loss contributed by the biofilm formation was attributable to the local fluid flows within the biofilm matrices themselves. It is well known that biofilms are formed in an aqueous phase and thus porous, consisting of 97% water structured with many microchannels and associated with cells, carbohydrates, lipids and biopolymers. When a seismic wave passes through the water-saturated and biofilm-filling sand, the biofilms can render extra squirt flows through their porous structures, which cause additional energy dispersion. The attenuation increases with an increase in biofilm quantity, as the internal squirt flow occurs more abundantly within biofilms. This biofilm-associated attenuation mechanism at low frequency contrasts with the energy loss mechanism at high frequency ($f > 10$ kHz), where the increased fluid–solid interfacial area by biofilm formation causes additional frictional loss in wave propagation (Noh *et al.*, 2016).

Summary and implication

In this study, RC tests were conducted on sand specimens containing EPS and biofilms formed from two bacteria *S. oneidensis* MR1 and *L. mesenteroides*. Both torsional and flexural excitation modes were applied to measure the changes in wave velocities V_s , V_f and the damping ratios D_s and D_f due to EPS growth.

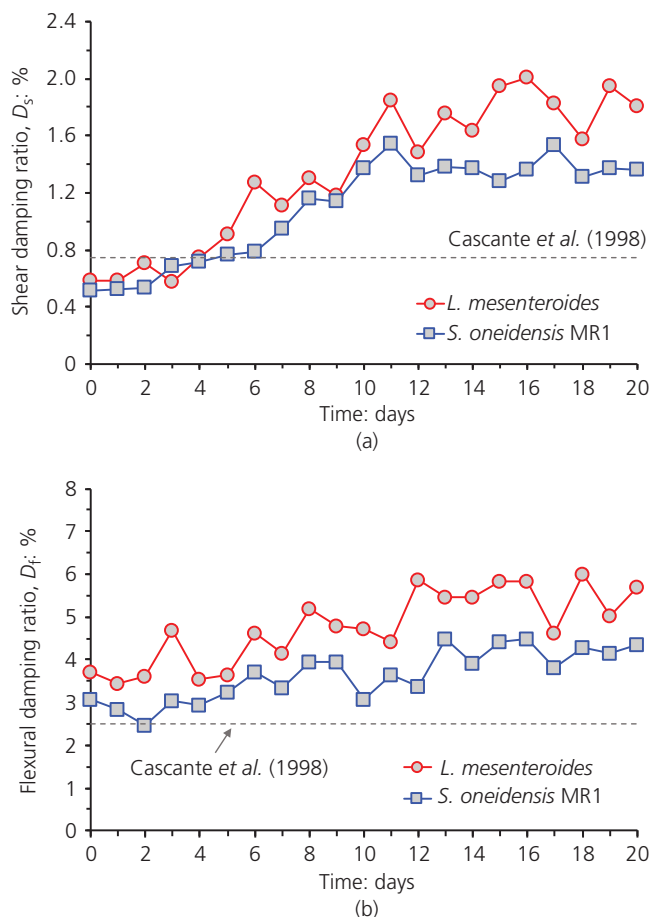


Figure 5. Changes in (a) shear damping ratio D_s and (b) flexural damping ratio D_f over the course of experiments during EPS formation by *L. mesenteroides* and *S. oneidensis* MR1. The D_s and D_f values of saturated Barco sands ($D_{50} = 0.44$ mm), measured using RC by Cascante *et al.* (1998), are superimposed for comparison with the current study's initial baseline measurements

The slight decreases in V_s and V_f indicate that EPS formation has a minimal effect on the wave velocities of saturated sand, suggesting that no appreciable increase in grain contact stiffness occurs. The damping ratios for both torsional and flexural mode, D_s and D_f , show significant increases due to the accumulation of EPS material in the specimens. Such increased attenuations are expected primarily due to the induced squirt fluid flows during wave propagation through porous media. The local fluid flows within the EPS matrices can cause additional energy dissipation and as a result, increase low-frequency attenuation.

This study presents unique and unprecedented experiment data on small-strain and low-frequency seismic responses, which clearly show an increased seismic attenuation with bacterial EPS formation in saturated sands. Although active microbial activities and their EPS production were confirmed through effluent analyses as well as post-experiment examination, the study lacks measurement on EPS quantities during the continuous feeds, which warrants further quantitative analyses on the correlation between EPS amount and low-frequency seismic attenuation.

These results imply that formation and accumulation of soft, viscous EPS or biofilms by bacterial activities can be detected by monitoring seismic attenuation and can alter the seismic attenuation responses of sands, such as the case under earthquake loading or blast-induced compaction. The frequency of the seismic surveys based on shear/surface waves, including cross-hole tomography, SASW/MASW, ranges from 10 to 100 Hz, therefore, the wave length ranges 2–20 m, assuming the shear wave velocity of 200 m/s. It is expected that the seismic survey would be capable of capturing the field-scale heterogeneity associated with EPS accumulation, if the anomaly scale is greater than the wave length by more than two times (e.g., ~8–80 m). This implies the in situ seismic tests would detect the EPS-associated heterogeneity on the scale of several meters to tens of meters.

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